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Juan M Esteve, Jean-Marie Launay, Odile Kellermann, Luc Maroteaux

FUNCTIONS OF SEROTONIN IN HYPOXIC PULMONARY VASCULAR REMODELING

Esteve Juan M[1], Launay Jean-Marie[3], Kellermann Odile[2], Maroteaux Luc[1,4]

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[1]IGBMC, F-67400 France ; INSERM, U596, Illkirch, F-67400 France ; CNRS UMR7104, Illkirch, F-67400 France ; Univ Strasbourg, Illkirch , F-67400 France.

[2]CNRS, UPR1983, Villejuif, F-94805 France

[3]Service de Biochimie, Hôpital Lariboisière, AP-HP, Paris, F-75010 France ; IFR139, EA3621, Paris, F-75010 France.

[4]INSERM, U616, Paris, F-75013 France ; Hop Pitié-Salpetrière, Paris, F-75013 France ; Univ Pierre et Marie Curie, Paris, F-75013 France.

Author for correspondence: Luc Maroteaux INSERM, U616; Hopital Pitié-Salpetrière; Université Pierre et Marie Curie Paris; Bat Pédiatrie, 47 Bd de l'Hopital 75013 Paris, France. TEL: (33) 01 42 16 26 73 FAX: (33) 01 45 70 99 90 Email: luc.maroteaux@chups.jussieu.fr

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Abstract

In lung vasculature, reversible constriction of smooth muscle cells exists in response to acute decrease in oxygen levels (hypoxia). Progressive and irreversible structural remodeling that reduces blood vessel lumen takes place in response to chronic hypoxia and results in pulmonary hypertension. Several studies have shown a role of serotonin in regulating acute and chronic hypoxic responses. In this review the contribution of serotonin, its receptors and transporter in lung hypoxic responses is discussed. Hypoxic conditions modify plasma levels of serotonin, serotonin transporter activity, and expression of 5-HT_{1B} and 5-HT_{2B} receptors. These appear required for pulmonary vascular cell proliferation, which depends on the ratio between reactive oxygen species and nitric oxide. A heterozygous mutation was identified in the 5-HT_{2B} receptor gene of a patient who developed pulmonary hypertension after fenfluramines anorexigen treatment. This C-terminus truncated 5-HT_{2B} mutant receptor presents lower nitric oxide coupling, and higher cell proliferation capacity than wildtype receptor. Under low oxygen tension, cells increase the transcription of specific genes via stabilization of the transcription factor HIF-1. Factors such as angiotensin II or thrombin that can also control HIF-1 pathway, contribute to pulmonary vascular remodeling. The 5-HT_{2B} receptor via phosphatidylinositol-3 kinase/Akt activates NF-kappaB, which is involved in the regulation of HIF-1 expression. A control of HIF-1 by 5-HT_{2B} receptors explains why expression of pulmonary vascular remodeling factors, such as endothelin-1 or TGF-beta, which is HIF-1-alpha regulated, is not modified in hypoxic 5-HT_{2B} receptor mutant mice. Understanding the detailed mechanisms involved in lung hypoxic responses may provide general insight into pulmonary hypertension pathogenesis.

Keywords: Arteries; Cell Division; Dexfenfluramine; Hypoxia; Physiopathology; Pulmonary Hypertension; Receptor; Serotonin; Transporter; Vascular Remodeling.

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Introduction

Aerobic evolution has resulted in mammalian cells and tissues metabolism that is oxygen-dependent. At normal O_2 tension, oxidative phosphorylation is the principal energy supply for eukaryotic cells but at low O_2 tension, metabolic switches turn off mitochondrial electron transport and activate anaerobic glycolysis. Maintenance of normal tissue function thus depends on a continuous supply of O_2, so it is crucial that the body detects and responds rapidly to hypoxia (1). The vascular system responds to acute hypoxia in several ways. In the systemic arterial system, acute hypoxia causes vasodilatation; in pulmonary arteries, however, it elicits reversible vasoconstriction (2). Unlike acute hypoxia, in response to chronic hypoxia, pulmonary vasculature remodeling (PVR) takes place upon persistent vasoconstriction, resulting in reduced blood vessel lumen diameter, increased resistance. This persistent vasoconstriction, when permanent becomes irreversible and causes pulmonary hypertension (PH) (3). PVR results from alterations of the balance between the effects of vasodilators and antiproliferative agents produced by the endothelium (*e.g.*, prostacyclin and nitric oxide (NO·), and vasoconstrictors and mitogenic factors (*e.g.*, endothelin-1, endothelium-derived growth factor, and 5-HT) (4-6). Understanding the mechanisms underlying irreversible PVR is an important step in defining therapeutic targets, since many known pulmonary vasodilators diminish pulmonary remodeling by reducing pulmonary pressure (7, 8), and remodeling of the walls of distal pulmonary arteries is common in secondary and primary PH (9).

Serotonin (5-hydroxytryptamine, 5-HT) mediates myriad functions in the nervous and vascular systems. In the central nervous system, 5-HT is synthesized by neurons in the raphe nucleus; in the periphery, 5-HT is produced by the enterochromaffin cells of the gut. The actions of 5-HT are mediated by four receptor classes: ligand-gated cation channels ($5\text{-}HT_3$ receptors) and three groups of G protein-coupled receptors ($5\text{-}HT_{1/5}$, $5\text{-}HT_2$, $5\text{-}HT_{4/6/7}$), each of which exhibits coupling to different in G proteins (10). The $5\text{-}HT_{1/5}$ receptor ($5\text{-}HT_1R$) subfamily has 7 members ($5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$, $5\text{-}HT_{1E}$, $5\text{-}HT_{1F}$, $5\text{-}ht_{5A}$, and $5\text{-}ht_{5B}$) that are negatively coupled to adenylyl

cyclase via pertussis toxin-sensitive $G_{i/o}$ proteins, leading to decreases in intracellular cAMP. The 5-HT_2R subfamily comprises 3 members (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) that are positively coupled to phospholipase C (PLC) via $G_{q/11}$ proteins, leading to increases in intracellular inositol 1,4,5-trisphosphate (IP_3), 1,2-diacylglycerol (DAG) and Ca^{2+}. The 5-HT_4, 5-HT_6, and 5-HT_7Rs are positively coupled to adenylyl cyclase via cholera toxin-sensitive G_s proteins, leading to increases in intracellular cAMP.

Several studies have demonstrated a role for 5-HTRs in regulating hypoxic responses. For instance, in the pond snail *Helisoma trivolvis*, a decrease in environmental O_2 levels after gastrulation stimulates cilia-mediated rotational movements of the embryo; this hypoxia-induced response is mediated by two serotonergic sensory-motor neurons that both detect reduced O_2 levels and activate ciliary movements (11). Also, intermittent hypoxia causes a long-term facilitation (LTF) of respiratory motor output; this neural plasticity requires 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2Rs (12). Furthermore, chronic hypoxia enhances LTF-evoked responses to intermittent hypoxia; this metaplasticity is mediated by 5-HT_2 and 5-HT_6 and/or 5-HT_7Rs (13). Different chemosensory organs such as the carotid bodies (CB) and pulmonary neuroepithelial bodies (NEB) respond to hypoxia in a serotonin-dependent fashion. CB type I cells contain 5-HT and express 5-HT_{1A}, 5-HT_3, and 5-HT_{5A}Rs that affect CB function when arterial pO_2 is reduced (14). NEBs release 5-HT in response to acute hypoxia by a mechanism involving the 5-HT_3R (15). Unlike hypoxic responses in the nervous system, which involve many different 5-HTR subtypes, hypoxia-induced vasoconstriction in the pulmonary vasculature appears to involve only 5-HT_1 and 5-HT_2Rs. In recent years, several studies have demonstrated that 5-HTRs control hypoxic responses in the pulmonary vascular system (16). The exact pathways through which hypoxia causes vasoconstriction and pulmonary vascular remodeling (PVR) are just beginning to be identified. What is clear, however, is that hypoxia alters molecular (*e.g.*, protein expression) and cellular (*e.g.*, proliferation) processes via mechanisms that involve serotonin, its receptors, and its transporter to elicit the physiological, pulmonary responses to hypoxia (vasoconstriction and PVR). In this

review, we will highlight the current understanding of the serotonin-dependent mechanisms underlying pulmonary hypoxic responses.

1. Regulation by 5-HT of hypoxia-induced PVR

1.1. Hypoxic conditions modify 5-HT levels

The function of 5-HTRs in hypoxic responses in the pulmonary vasculature must be dependent on the presence of suitable 5-HT levels activating these receptors. In healthy subjects, unconjugated plasma 5-HT levels are low (<10 nM); however, in PH patients, plasma 5-HT is consistently elevated (17-19). A deficiency in platelet 5-HT storage, as is characteristic of Fawn hooded rats, contributes to the development of severe PH under both normoxic (20) and hypoxic (high altitude) (21) conditions. These observations suggest an etiological role for 5-HT in the development of PH and raise two important questions: 1) what is the source of 5-HT in the pulmonary vasculature, and 2) how does reduced O_2 lead to an increase in plasma 5-HT levels?

In the periphery, 5-HT is synthesized and secreted from neuroendocrine enterochromaffin cells in the gut. 5-HT is mainly eliminated by uptake in lung endothelial cells, where it is then degraded by MAO (22). Platelets take up 5-HT through the 5-HT transporter (5-HTT) and store—but only slowly degrade—the monoamine. Former studies have shown that long-term hypoxia causes decreased platelet counts and short-term hypoxia increased platelet counts (23). Later, it has been established that chronic hypoxia, a stimulator of erythropoiesis, causes thrombocytopenia in laboratory animals. The thrombocytopenia is most likely the result of a reduction in the production of platelets caused by a decrease in the number of megakaryocytes in the bone marrow. The thrombocytopenia seems to be caused by competition of a precursor cell of the erythrocytic and megakaryocytic cell lines (24). Moreover, hypoxia facilitates platelets aggregation (25). Alteration of platelet number and/or function under hypoxic conditions could thus concertedly reduce 5-HT uptake and would explain hypoxia-induced increases in circulating plasma 5-HT. In this regard, platelet activation was found in the pulmonary vessels of patients with PH secondary to chronic

obstructive pulmonary disease (26), and platelet survival time is reduced in patients with hypoxemia and PH (27). Anti-platelet agents, such as dipyridamole, reduce hypoxemic PH and the thickness of pulmonary arteries in response to chronic hypoxia (28). Based on these results, it has been postulated that circulating plasma 5-HT may originate from platelets (29).

Pulmonary NEB also secrete 5-HT in response to airway hypoxia (15). In this way, cellular and molecular hypoxia-regulated mechanisms, which have an effect on circulating plasma 5-HT levels, probably involve platelets and pulmonary NEB, as well as reductions in the lungs' ability to uptake and/or remove 5-HT. The 5-HT_{2A}Rs have been detected in platelets (30, 31), where they enhance platelets aggregation (25). The activation of presynaptic 5-$HT_{1B/1D}$R decreases 5-HT release (32), and in neonatal rabbit pulmonary NEB, 5-HT_3Rs are involved in a positive feedback loop resulting in hypoxia-induced 5-HT release (15). Together these observations suggest that 5-HT receptors control plasma levels of their ligand in response to hypoxia.

1.2. Putative role of 5-HTT in the hypoxic PVR

In recent years, many studies have explored the possible role of 5-HTT in hypoxia-induced PVR. Hypoxia causes changes in 5-HTT expression: acute and chronic hypoxia increase 5-HTT mRNA levels in rat pulmonary arteries (33). Upon acute hypoxia, specific 5-HT transport is increased in porcine pulmonary artery endothelial cells without a concomitant increase in K_m; Acute hypoxia (i) results in an elevation of the maximal uptake rate (V_{max}), implying *de novo* protein synthesis, and (ii) modifies plasma membrane phospholipids and consequently its fluidity (34). Conversely, chronic hypoxia reduces 5-HT uptake by pulmonary arteries (35).

In rat pulmonary artery SMC, 5-HT induces DNA synthesis, and acute hypoxia potentiates this mitogenic effect. The increase in DNA synthesis can be prevented by high concentrations of 5-HTT inhibitors (36). In mice, increased PVR as a result of exposure to chronic hypoxia is partially reduced by the 5-HTT inhibitors citalopram and fluoxetine (37). Nonetheless, in sodium-free conditions (*i.e.*, without 5-HT uptake), 5-HTT inhibitors still attenuated 5-HT-induced mitogenesis (38). Importantly, some 5-HTT inhibitors (including citalopram and fluoxetine) have μM affinities

for 5-HT_2R (39). Recent results indicate that there is synergy between the inhibitory effects of 5-HT_{1B}R antagonists and 5-HTT inhibitors on 5-HT-induced pulmonary vasoconstriction (40) and that nordexfenfluramine (NorDF)-induced vasoconstriction is not dependent on 5-HTT-mediated release of endogenous 5-HT but rather via direct activation of 5-HTRs (41). These observations suggest that 5-HT uptake by 5-HTT cannot fully account for the proliferative action of 5-HT, and support a role for 5-HTRs. The proposition that the long 5-HTT promoter polymorphism promotes PVR through increased 5-HTT expression does not fully explain why patients who develop PH after dexfenfluramine (DF) treatment have the same proportion of this polymorphism as do PH patients in general (42) (Launay unpublished). Moreover, the report that PVR after chronic hypoxia is reduced—but not completely abolished—in mice deficient for 5-HTT gene (43) demonstrates that 5-HTT does not solely mediate hypoxia-induced PVR.

1.3. Regulation of hypoxia-induced PVR by 5-HTRs

Different mechanical factors have been shown to induce PVR. Chronic hypoxia can stimulate PVR directly and/or by a persistent vasoconstriction process as already suggested (44). Despite sustained hypoxia, vasoconstriction persists but subsides somewhat as PVR progresses (7). Neurohumoral factors such as 5-HT/5-HTRs may be implicated.

The 5-HT_{1B}R-mediated contractile response to 5-HT or 5-carboxamidotryptamine is increased in pulmonary arteries isolated from chronic hypoxic wild-type mice. However, the activity of 5-HT_{1B}R does not seem to be limiting, as 5-HT_{1B}R knockout mice still respond to hypoxia but develop less severe PH and PVR than do wild-type mice (45). Discordantly, Marcos et al. report that chronic hypoxia (10% O_2 for 2 weeks)-induced pulmonary hypertension and increased vessel muscularization were not reduced by the 5-$HT_{1B/1D}$R antagonist GR127935 (37). Thus, the role of 5-HT1BR in hypoxia-induced PH and PVR remains unclear and may be species- or strain-sensitive.

In ovine common carotid arteries, despite altering the contractile response, acute hypoxia had no effect on 5-HT_{2A}R coupling to IP_3 second-messenger production (46). Similarly, acute

hypoxia reduced 5-HTR density and agonist affinity in adult bovine common carotid arteries (47). However, the role of 5-HT_{2A}R in hypoxia-induced PH and PVR is not clear, since the receptor's expression is not modified in the lung vasculature of mice exposed to 10% O_2 for 5 weeks (48). Furthermore, in mice, the effects of chronic hypoxia on pulmonary artery pressure and vessel muscularization are insensitive to the 5-HT_{2A}R antagonist ketanserin (37).

Interestingly, mice with pharmacologically or genetically inactive 5-HT_{2B}R do not develop PH and PVR following chronic hypoxia, even though the acute hypoxic response (vasoconstriction) is intact (48). Therefore, the 5-HT_{2B}R is a key factor in the molecular signaling pathways that couple chronic hypoxia to PH and PVR, a pathway independent of acute hypoxia-induced vasoconstriction. The 5-HT_{2B}R also functionally interacts with the 5-HT_{1B}R and the 5-HTT, whose roles in PH and PVR are rather well established. For instance, 5-HT_{1B}R and 5-HTT activities are modulated by 5-HT_{2B}Rs (29, 49). Similarly, MacLean proposed a functional interaction between G_i-coupled (5-HT_{1B}R) and the 5-HT transporter, which would facilitate the development of PH (40). In addition, 5-HTT, 5-HT_{1B}R, and 5-HT_{2B}R are colocalized in pulmonary arteries, and 5-HT_{2B}R has been reported to regulate 5-HTT activity in the 1C11 serotonergic cell line (50). The emerging question, then, is how 5-HTRs control hypoxia-induced PVR.

1.4. Possible mechanisms relating vascular injuries to 5-HTRs

Hypoxia changes levels of reactive oxygen species (ROS) and NO· in the pulmonary vascular wall, and these alterations are involved in PVR (51). ROS and NO· levels are sensitive to 5-HTR activity. For example, 5-HT_{1B}Rs and 5-HT_{2B}Rs have been shown to increase NO· levels in human coronary artery endothelial cells (52), and and to elicit relaxation in porcine pulmonary arteries via the release of NO (53). In addition, the 5-HT_{2A}R induces NO· release by regulating gastrointestinal transit in mice (54), but can inhibit cytokine-stimulated inducible NO· synthase in C6 glioma cells (55). In the cerebral vasculature, NO· release from endothelial cells occurs following activation of 5-HT_{2B}Rs (56). In several cell lines, 5-HT_{2B}Rs have been shown to activate both constitutive and inducible NO· synthases via interactions requiring the receptor's C-terminal

PDZ binding domain (57). In this way, 5-HT_{2B}R activity leads to NO· generation both *in vitro* and *in vivo*.

In renal mesangial cells, 5-HT_{2A}R activation induces ROS (*e.g.*, H_2O_2 and superoxide) production via an NAD(P)H oxidase-like enzyme (58). In the hippocampus of mice lacking the 5-HTT, increased DNA oxidation has been observed, suggesting that the transporter serves as an anti-oxidant. However, 5-HTT null mice do not exhibit alterations in glutathione (GSH), oxidized glutathione (GSSG), and other anti-oxidant systems (59). Recently it has been reported that 5-HT_{2B}R activation can also induce ROS production by a NAD(P)H oxidase-dependent mechanism in a serotonergic cell line (60). The mitochondrial electron transport chain is a major ROS source, and mitochondria are a target of 5-HT_{2B}R anti-apoptotic signaling in cardiomyocytes (61). Thus, 5-HT_{2B}R signaling leads to changes in both NO· and ROS production.

1.5. Mutation in 5-HT_{2B}R gene and PH in human exposed to DF

Recently, by investigating the 5-HT_{2B}R gene in patients who developed pulmonary hypertension after intake of DF, a heterozygous mutation was found in one female patient who followed a nine-month anorexigen regimen (62). The polymorphism, R393X, results in a truncation of the receptor's C-terminal tail, thus removing (i) putative palmitoylation and phosphorylation sites essential for internalization, and (ii) the PSD-95, Dlg, ZO-1 (PDZ) binding motif involved in the coupling to NOS and other scaffold proteins. Functionally, the R393X 5-HT_{2B}R exhibits a loss of rapid internalization compared to the wild type receptor, a finding consistent with removal of C-terminal determinants of receptor trafficking (63). In addition, the R393X 5-HT_{2B}R displays diminished coupling to NOS compared with the wild type receptor, as expected from removal of the PDZ binding motif. Despite the apparent losses of function due to the R393X polymorphism, the truncated 5-HT_{2B}R variant displays striking gain of function *vis-à-vis* proliferative capacity, an effect that appears to result from a switch from wild type dual G_q/G_{13} coupling to a nearly exclusive G_{13} coupling. Thus, given the role of cell proliferation in PH and PVR, the R393X 5-HT_{2B}R polymorphism is clearly relevant for vascular proliferation and remodeling (63). The α subunit of

G_{13} plays a critical role in 5-HT_{2B}R-NOS coupling, (63) and G_{13} has been reported to activate inducible NOS through a mechanism distinct from that other G_{α} isoforms (64). Src family kinases (effectors of 5-HT_{2B}Rs) (65) act upstream of the small G-protein Rho in $G_{12/13}$-induced JNK activation (66). RhoA and its effector Rho kinase play a major role in the effects of both acute and chronic hypoxia on the pulmonary circulation, possibly by modulating both vasoconstriction and vascular remodeling (67). The increased G_{13} coupling displayed by the R393X 5-HT_{2B}R likely renders the polymorphism relevant to pathological vasoconstriction and remodeling in response to chronic exposure to DF (Fig. 1).

2. Control of hypoxia-dependent transcription by 5-HT

Under low oxygen tension, cells increase the transcription of genes involved in angiogenesis, erythropoiesis, and glycolysis.

2.1 Transcriptional regulation of 5-HT-related molecules

Hypoxia causes transcriptional regulation of both the 5-HTT and 5-HTRs. For example, 5-HT_{1B}R and 5-HT_{2B}R mRNA levels are increased in the lung vasculature of mice exposed to chronic hypoxia (68), while 5-HT_{2A}R expression is unaffected (48). Rat 5-HTT mRNA expression is stimulated in proximal pulmonary arteries and lungs upon chronic hypoxia (33). In humans, DF use for periods greater than three months is associated with an increased risk for developing PH (6, 69). DF, a known substrate of 5-HTT, is metabolized *in vivo* by N-de-ethylation in norDF, which is a potent and selective 5-HT_{2B}R agonist (70, 71). While DF does not trigger PH in mice after 5 weeks under normoxic conditions, administration of the drug does potentiate hypoxia-induced PH, (48) suggesting that hypoxia (like norDF) acts directly on 5-HTRs to contribute to their actions in the pulmonary vasculature. Together, these observations raise the question whether increased expression of 5-HTRs is a prerequisite for PVR, and/or if elevation of 5-HT levels and subsequent activation of basal 5-HTRs can induce PVR in absence of a hypoxic stimulus.

2.2 Regulation of cell proliferation by 5-HT molecules

Hypoxia-induced pulmonary artery vascular cell proliferation triggers PVR, which increases pulmonary artery pressure (72). *In vitro*, phenotypically distinct SMC subpopulations in the media of bovine main pulmonary artery display differential proliferative responses under extreme hypoxic (3% O_2 during 72 hours) conditions: DNA synthesis is increased only in a subset of the medial subpopulations (73). In bovine and rat pulmonary artery SMC, 5-HT induced DNA synthesis (74), and 24 hours under hypoxic conditions can potentiate 5-HT's mitogenic effect (33). Serotonin causes proliferation of many cell types in culture, including vascular SMC, via not only 5-HTRs but also through crosstalk with other signal transduction systems, such as the receptors for the growth factors platelet-derived growth factor (PDGF), fibroblast growth factor, and epidermal growth factor (75). Hypoxia inhibits the release of anti-mitogenic factors such as prostacyclin in cultured pulmonary artery endothelium (76). The absence of hypoxia-induced thymidine incorporation (a measure of cell proliferation) and vascular muscularization in the lungs of hypoxic 5-$HT_{2B}R^{-/-}$ mice indicates an absolute requirement for 5-HT_{2B}R in chronic hypoxia-induced mitogenesis. Further demonstrating the key role of 5-HT_{2B}Rs in hypoxia-induced mitosis is the finding that the highly selective 5-HT_{2B}R antagonist RS-127445 abrogates proliferation and PVR in wild-type mice exposed to hypoxia (48).

Activation of MAPK, another mediator of proliferative signals, has also been implicated in PVR (44). PDGF causes proliferation in pulmonary arterial SMC (77) and increased expression of PDGF has been reported in rat lungs subsequent to hypoxic PH (78). In mouse fibroblast $LMTK^-$ cells stably expressing murine 5-HT_{2B}Rs, 5-HT-induced receptor activation leads to cell cycle progression via a complex signaling pathway in which the cytoplasmic tyrosine kinase c-Src controls cyclin E expression and, in concert with PDGF receptor transactivation, induces cyclin D1 expression in a MAPK-dependent fashion (65).

In sheep with hypoxia-induced PH, increased mRNA levels of the transforming growth factor-β (TGF-β) have been associated with PVR (79). It has been suggested that interleukins (ILs)

and tumor necrosis factor (TNF)-α are also involved in the development of PVR: serum concentrations of IL-1 and IL-6 are increased in patients with primary PH (80); IL-1 is a mitogenic factor in human and rat vascular SMC (81); hypoxia stimulates IL-1 production in human vascular SMC (82), and NF-IL6 activates IL-6 gene transcription in hypoxic pulmonary vascular endothelial cells (83). Chronic hypoxia (10% O_2 for 5 weeks) also causes increased TGF-β levels lung vessel culture supernatants via a mechanism that requires 5-HT_{2B}R activity (48). The release of TNF-α, IL-1β, and IL-6 in response by isoproterenol-treated cardiac fibroblasts is also 5-HT_{2B}R-dependent (84).

Matrix metalloproteinases (MMPs) activation and extracellular matrix (ECM) remodeling contribute to hypoxia-induced pulmonary vascular proliferation in PVR. Some of the best characterized ECM substrates for MMPs are collagens, elastin, and proteoglycans (9). An increase in elastase activity leads to the release of latent growth factors (85) and elastase activity is increased in mice with chronic hypoxia-induced PH (86), an effect that is absent upon genetic or pharmacological ablation of 5-HT_{2B}Rs (48). These observations suggest that 5-HT_{2B}R-dependent elastase activity leads to latent growth factor release including TGF-β. In addition, collagenolytic activity in extracts from pulmonary arteries in rats exposed to hypoxia is increased (87) that may include MMP activity contributing to PVR. A possible role for 5-HT_{2B}R in the activation of various MMPs in PVR is likely, given that activation of MMP-2 (ECM substrates: various collagens, fibronectin, laminin, aggrecan, insoluble elastin) and MMP-9 (ECM substrates: various collagens) is regulated by 5-HT_{2A}Rs in uterine tissue (88) and by 5-HT_{2B}Rs in cardiac fibroblasts (LM unpublished). Recently, the 5-HT_{2B}R was shown to stimulate TNF-α converting enzyme in a serotonergic cell line (60). Thus, 5-HT_2R appears to be crucial for hypoxia-induced PVR via MMP-regulated growth factor expression in the lung vasculature (Fig. 2).

3. Regulation of transcription factors expression by 5-HT molecules

Hypoxic transcription processes are coordinately regulated by the hypoxia-inducible factor HIF-1, which, despite its constitutive expression, is only active in response to reduced O_2 levels (89). Several line of experimental evidence support the hypothesis that factors controlling HIF-1 expression also contribute to hypoxia-induced PVR. Indeed, a strong correlation has been found between HIF-1α overexpression and immunoreactivity of the cell proliferation index Ki67 (90). Though hypoxia is the ubiquitous inducer of HIF-1α, other stimuli, such as insulin, insulin-like growth factors 1 and 2, and EGF, also increase HIF-1α protein levels in some cells. These stimuli also induce VEGF expression in an HIF-1-dependent manner.

3.1 Putative regulation of HIF molecules by 5-HT

The O_2-regulated transcription factor HIF-1 has been proposed to control the expression of several agents involved in PVR, such as endothelin-1 and TGF-β (91). HIF-1α hypomorphic mice develop less severe medial wall thickening in the pulmonary arterioles than do wild type mice maintained for 3 weeks at 10% O_2 (92). Other factors, such as angiotensin II (Ang II), thrombin, platelet-derived growth factor, can increase HIF-1α in vascular SMC to levels beyond those resulting from hypoxic treatment. The non-hypoxic induction of the HIF-1 transcription factor via vasoactive hormones (Ang II and thrombin) is triggered by a dual mechanism, *i.e.*, PKC-mediated transcriptional activation and ROS-dependent increases in HIF-1α protein expression (93). In 5-HT_{2B}R-expressing cells, receptor activation increases the activity of the c-Src family tyrosine kinase and ROS levels through NAD(P)H oxidase (60, 65), which can thus induce HIF-1α expression.

3.2 Hypoxia-induced NF-κB expression by 5-HT_{2B}Rs

In cardiomyocytes, 5-HT_{2B}Rs, via phosphatidylinositol-3 kinase/Akt, activate NF-κB, an event that is required for the receptors' anti-apoptotic effects (61). Activation of NF-κB is sufficient to suppress cell death of ventricular myocytes during hypoxia. Additionally, NF-κB averts cell death through a mechanism that prevents perturbations to the mitochondrion during hypoxic injury (94). Moreover, the 5-HT_{2B}R has recently been identified in a large-scale screen for human genes

that activate NF-κB signaling pathways (95). Furthermore, treatment with LY294002 (a selective inhibitor of phosphatidylinositol 3-kinase) significantly inhibits erythropoietin protein and mRNA expression in Hep3B cells exposed to hypoxia for 24 hours. Inhibition of NF-κB with a super-repressor (dominant negative IκBα) also significantly blocks HIF-1 transactivation, as well as erythropoietin gene expression [129]. We propose that 5-HT_{2B}R expression, which is increased under hypoxic conditions, is a trigger of HIF-1α via phosphatidylinositol-3 kinase/Akt/NF-κB pathway.

3.3 Hypoxia-induced NF-κB may regulate HIF expression

Putative control of HIF-1 by 5-HT_{2B}Rs would explain why the expression of PVR-inducing factors, such as endothelin-1 and TGF-β (both of which are HIF-1-regulated), is not modified in hypoxic 5-$HT_{2B}R^{-/-}$ mice. A recent study by Moncada's group (96) showed that upon hypoxia, inhibition of mitochondrial respiration by NO· leads to a redistribution of intracellular O_2 toward other O_2-dependent targets, such as prolyl hydroxylase, which causes the constitutive degradation of HIF-1α. Stimulation of the NO/PKG pathway in rats treated with sildenafil increases RhoA protein levels, phosphorylation, and association with RhoGDI in the pulmonary artery, effects opposite to those induced by chronic inhibition of NO· synthesis or hypoxia. The observed NO· alterations in the hypoxic pulmonary vascular wall lead to a plausible explanation for the absence of hypoxia-induced PVR in 5-$HT_{2B}R^{-/-}$ mice and further strengthen the hypothesis that 5-HT_{2B}R expression under hypoxic conditions is not a target but a trigger of HIF-1 (Fig. 3).

Pressing Questions

Determining of the contribution of 5-HT to hypoxic responses in lung endothelial cells, smooth muscle cells, and fibroblasts is of major importance. How and when do these different cell types participate in the development and/or progression of PH? Of particular interest is the origin of the process. Do normoxic endothelial, fibroblast, and /or smooth muscles cells respond to 5-HT in a similar way as hypoxic cells? When does irreversible commitment to hypoxia-induced PVR occur? Given that most studies have focused on lung vasoconstrictive factors, how closely does

development of the pathology follow that of vasoconstriction? Finally, is the developmental program that generates lung vessels in the embryo retained in the lung vasculature of the adult? Is this program reactivated in adult lung pathophysiology? The answers to these questions will provide general insights into the pathogenesis of PH and may suggest novel therapeutic approaches to treating lung diseases in humans.

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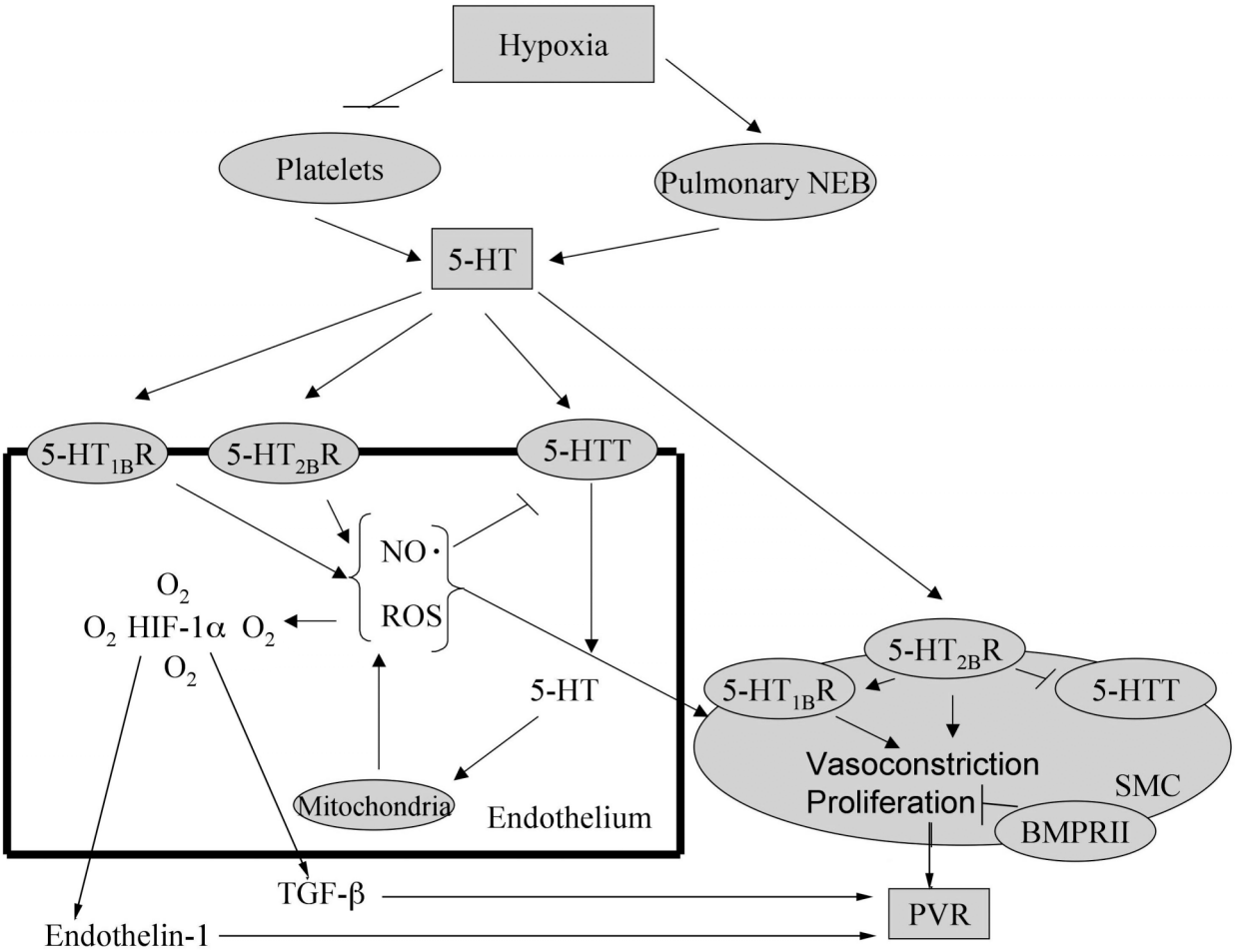

Figure 1. 5-HT in hypoxia-induced PVR. Hypoxia raises 5-HT levels by operating at platelets and pulmonary NEB, which acts at 5-HT_{2B}R, 5-HT_{1B}R and 5-HTT. Changes in ROS and NO· levels upon hypoxia, result in endothelium and vascular SMC oxidative damage and death. ProPVR agents, such as endothelin-1 and TGF-β, have no effect on PVR in the absence of 5-HT_{2B}Rs. Lines with arrows indicate positive actions and with a T negative effects.

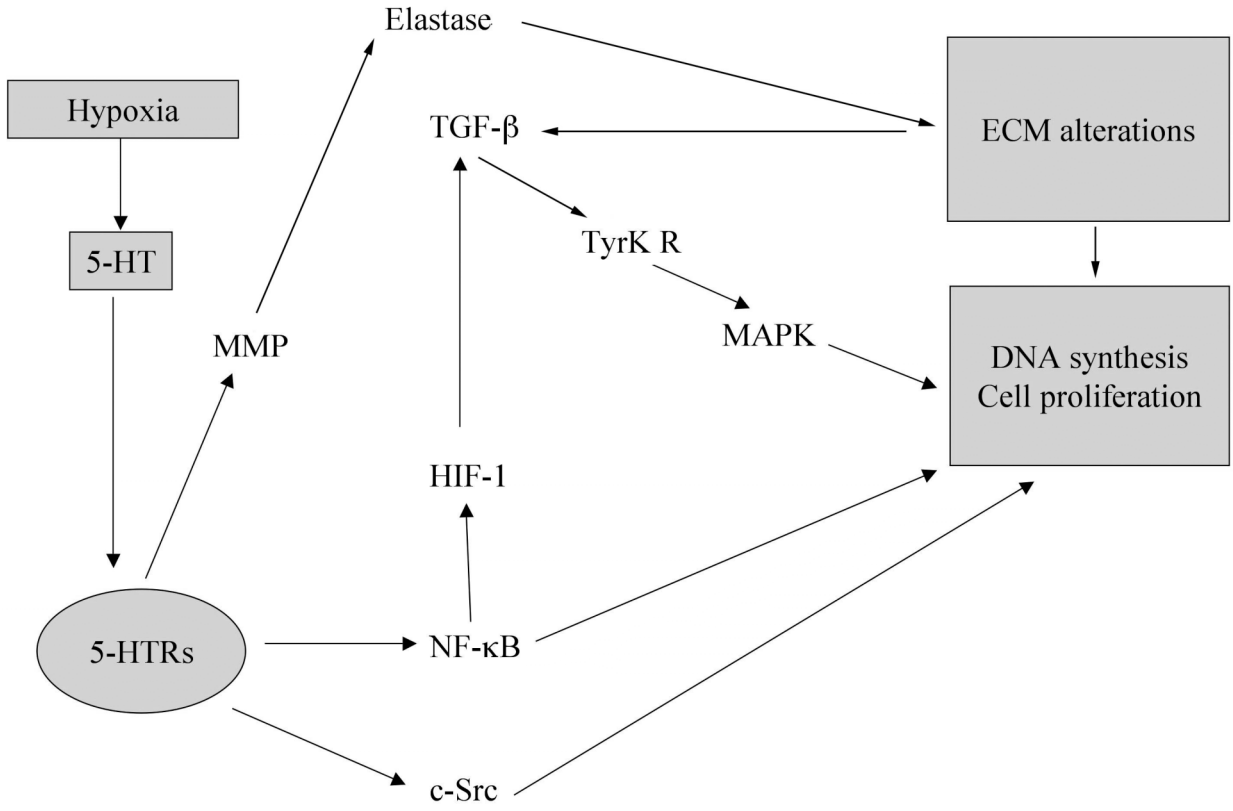

Figure 2. 5-HT-stimulated transcription pathways in the regulation of hypoxia-induced PVR. By controlling 5-HT levels, hypoxia has effects on transcriptional and post-transcriptional control of growth factors, via proteinases, which participate in ECM maintenance, leading to PVR.

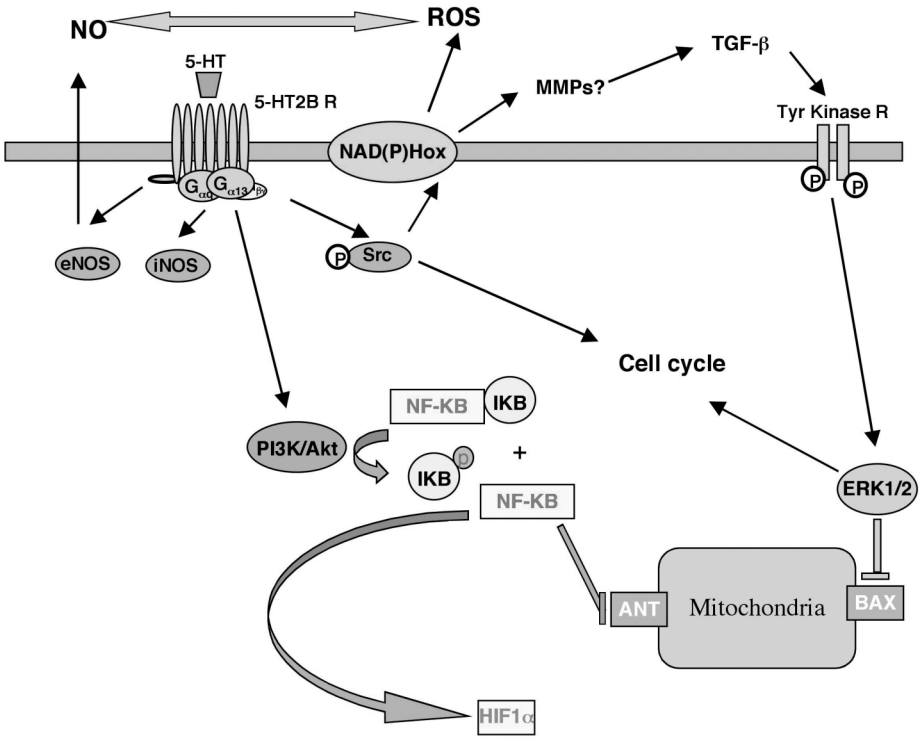

Figure 3. 5-HT_{2B}R-induced transduction pathways relevant to pulmonary hypertension. Different experimental evidences support a model in which ROS and NO· can be controlled by 5-HT_{2B}R that would result in transcriptional activation of cell cycle and hypoxic responses.